

Claims

What is claimed is:

1. A composition comprising a human skin equivalent, said skin equivalent having a surface electrical capacitance of from about 40 to about 240 pF.
2. The composition of Claim 1, said human skin equivalent having a surface electrical capacitance of from about 80 to about 120 pF.
3. The composition of Claim 1, wherein the combined content of ceramides 5, 6, and 7 in said skin equivalent is from about 20 to about 50% of total ceramide content.
4. The composition of Claim 1, wherein the content of ceramide 2 in said skin equivalent is from about 10 to about 40% of total ceramide content.
5. The composition of Claim 1, wherein said skin equivalent comprises keratinocytes selected from the group consisting of primary keratinocytes and immortalized keratinocytes.
6. The composition of Claim 5, wherein said immortalized keratinocytes are NIKS cells.
7. The composition of Claim 5, wherein said keratinocytes express heterologous GKLF.
8. The composition of Claim 1, further comprising keratinocytes derived from two different sources.

9. Isolated keratinocytes comprising a DNA construct comprising a sequence encoding GKLf operably linked to an exogenous promoter.
10. An organotypic culture comprising the keratinocytes of Claim 9.
- 5 11. A method of making skin equivalents having improved barrier function comprising:
- a) providing keratinocytes and a culture media comprising ascorbic acid and linoleic acid;
 - 10 b) culturing said keratinocytes under conditions such that a skin equivalent having improved barrier function is formed.
12. The method of Claim 11, wherein said ascorbic acid is provided at concentration of from about 10 to 100 micrograms/ml.
- 15 13. The method of Claim 11, wherein said ascorbic acid is provided at a concentration of about 0.05 mg/ml.
14. The method of Claim 11, wherein said linoleic acid is provided at a concentration of from about 5 to 80 micromolar.
- 20 15. The method of Claim 11, wherein said keratinocytes are selected from the group consisting of primary and immortalized keratinocytes.
- 25 16. The method of Claim 15, wherein said immortalized keratinocytes are NIKS cells.
17. The method of Claim 11, wherein said keratinocytes contain a DNA construct comprising a sequence encoding GKLf operably linked to an inducible promoter.

18. The method of Claim 11, wherein said skin equivalent has a surface electrical capacitance of from about 40 to about 240 pF.
19. The method of Claim 18, wherein said skin equivalent has a surface electrical capacitance of from about 80 to about 120 pF.
20. The method of Claim 11, wherein the content of ceramides 5, 6, and 7 in said skin equivalent is from about 20 to about 50% of total ceramide content.
21. The method of Claim 11, wherein the content of ceramide 2 in said skin equivalent is from about 10 to about 40% of total ceramide content.
22. The skin equivalent produced by the method of Claim 11.
23. A method of making skin equivalents having improved barrier function comprising:
- a) providing keratinocytes and a DNA construct comprising a sequence encoding GKLf operably linked to an exogenous promoter;
 - b) transfecting said keratinocytes with said DNA construct to provide transfected keratinocytes; and
 - c) culturing said transfected keratinocytes under conditions such that a skin equivalent having improved barrier function is formed.
24. The method of Claim 23, wherein said culturing step comprising culturing said transfected keratinocytes in a culture media comprising ascorbic acid and linoleic acid.
25. The method of Claim 24, wherein said ascorbic acid is provided at concentration of from about 10 to 100 micrograms/ml.

26. The method of Claim 24, wherein said ascorbic acid is provided at concentration of from about 50 micrograms/ml.
27. The method of Claim 24, wherein said linoleic acid is provided at a concentration of from about 5 to 80 micromolar.
28. The method of Claim 23, wherein said keratinocytes are selected from the group consisting of primary and immortalized keratinocytes.
29. The method of Claim 28, wherein said immortalized keratinocytes are NIKS cells.
30. The method of Claim 23, wherein said keratinocytes contain a DNA construct comprising a sequence encoding GKLF operably linked to an inducible promoter.
31. The method of Claim 23, wherein said skin equivalent has a surface electrical capacitance of from about 40 to about 240 pF.
32. The method of Claim 23, wherein said human skin equivalent having a surface electrical capacitance of from about 80 to about 120 pF.
33. The method of Claim 23, wherein the content of ceramides 5, 6, and 7 in said skin equivalent is from about 20 to about 50% of total ceramide content.
34. The method of Claim 23, wherein the content of ceramide 2 in said skin equivalent is from about 10 to about 40% of total ceramide content.
35. The skin equivalent produced by the method of Claim 23.
36. A method of screening compounds comprising

- a) providing a skin equivalent having a surface electrical capacitance of from about 40 to about 240 pF,
- b) treating said skin equivalent with said compound.

5 37. The method of Claim 36, further comprising step c) assaying an effect of said compound on said skin equivalent.

38. The method of Claim 36, wherein said compound is selected from a combinatorial library.

10 39. The method of Claim 36, wherein said human skin equivalent has a surface electrical capacitance of from about 80 to about 120 pF.

15 40. The method of Claim 36, wherein the content of ceramides 5, 6, and 7 in said skin equivalent is from about 20 to about 50% of total ceramide content.

41. The method of Claim 36, wherein the content of ceramide 2 in said skin equivalent is from about 10 to about 40% of total ceramide content.

20 42. The method of Claim 36, wherein said skin equivalent comprises keratinocytes selected from the group consisting of primary keratinocytes and immortalized keratinocytes.

43. The method of Claim 42, wherein said immortalized keratinocytes are NIKS cells.

25 44. The method of Claim 36, wherein said keratinocytes express heterologous GKLf.

30 45. A kit comprising at least one skin equivalent having a surface electrical capacitance of from about 40 to about 240 pF.

46. The kit of Claim 45, further comprising culture media for culturing said at least one skin equivalent.
47. The kit of Claim 45, further comprising instructions for culturing said skin equivalent.
48. The kit of Claim 45, further comprising instructions for testing compounds using said at least one skin equivalent.
49. The kit of Claim 45, wherein said skin equivalent having a surface electrical capacitance of from about 80 to about 120 pF.
50. The kit of Claim 45, wherein the content of ceramides 5, 6, and 7 of said skin equivalent is from about 20 to about 50% of total ceramide content.
51. The kit of Claim 45, wherein the content of ceramide 2 of said skin equivalent is from about 10 to about 40% of total ceramide content.
52. The kit of Claim 45, wherein said human skin equivalent comprises keratinocytes selected from the group consisting of primary keratinocytes and immortalized keratinocytes.
53. The kit of Claim 51, wherein said immortalized keratinocytes are NIKS cells.
54. The kit of Claim 45, wherein said keratinocytes express heterologous GKLF.